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A HISTOMORPHOMETRIC ANALYSIS OF THE CROSS-FACIAL NERVE GRAFT IN THE TREATMENT OF FACIAL PARALYSIS

ABSTRACT

One of the most unsettling sequela of facial paralysis (FP) is the loss of the blink reflex, leading to both a functional and aesthetic deformity. A successful method of treating FP and, in particular, loss of eye-sphincter function, is the use of the cross-facial nerve graft (CFNG) to reinnervate the previously denervated orbicularis oculi muscle. The present study examined the histomorphometric aspects of the entire CFNG, with respect to axon diameter and myelin area. The axon profile of the CFNG had a positive correlation with motor end-plate counts and electrophysiologic recordings. These results should help in further understanding the number of motor axons needed to restore adequate function to the paralyzed eye sphincter, and establish more rational reconstructive procedures.

Facial paralysis (FP) may arise from a variety of causes, including ischemia, infection, trauma, compression, and erosion by tumor. Without a doubt, the most serious result of VIIth nerve paralysis is the loss of the blink reflex, which invariably leads to conjunctival desiccation and abrasion, infection of the cornea, and eventually blindness.[1]

Before considering the various treatment strategies for FP, it is important to discuss several significant factors that play a role in our model and, specifically, the degree of regeneration and the recovery of function. The success of regeneration, with axons reaching appropriate end organs (motor end-plates) is affected by the distance between the site of injury and the muscle target. Regeneration is more effective over shorter distances.[2] In addition, the presence of anatomic discontinuity or scar tissue at the repair site may inhibit regeneration and hinder recovery of function.[3] Moreover, the issue of timing is an important consideration in the treatment of FP. If primary repair is possible, the surgeon has an increased possibility of achieving exact orientation of the cut nerve ends.[4,5]

The direct neurotization technique,[4–10] which involves the implantation of the proximal stump of a transected nerve into a denervated muscle, is applicable only in cases where the surgeon wishes to strengthen a weak target without sacrificing its normal innervation.

Nerve transfer or nerve cross-over involves transferring the proximal stump of a nerve to the distal stump of another more important nerve. The nerve-transfer technique has been described at the beginning of this century, using the hypoglossal,[11–15] accessory,[16–18] and phrenic nerves[19] as motor donors. However, significant drawbacks remain, including uncontrollable mass movement, synkinesis of the eye sphincter with the lower face, absence of emotional expression, and unwanted contractures.

Where the length of the nerve deficit precludes

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direct repair, restoration of eye-sphincter function is achieved through the utilization of a nerve graft.[20–23] Success is contingent on the presence of a healthy, well-vascularized, and unscarred recipient bed.[24,25]

The cross-facial nerve-graft (CFNG) technique involves the use of relatively long nerve grafts as conduits to carry axons from the normal to the paralyzed side of the face.[26–28] A CFNG, when used as the sole treatment, is most effective when denervation time is 6 months or less. In the past, choice of the donor nerves varied between the buccal branch,[29] buccal and zygomatic branch,[30] and the cervical branch.[29] Depending on whether the paresis is global or partial, the senior author (JKT) uses selected components of the appropriate branches of the facial nerve on the normal side, using a technique that involves extensive intraoperative microstimulation and mapping of motor territories of each branch, followed by selective neurectomies and microcoaptation with the corresponding CFNG.

One of the most disturbing sequela of VIIth nerve paralysis is the loss of the blink reflex, which results in both a functional and aesthetic deformity. In the past, the loss of the blink reflex was ignored. Kugelburg[31] first addressed the absence of the blink reflex in the paralytic face, using electromyographic recordings of the orbicularis oculi muscle.

Recently, our laboratory[32] established a blink-reflex model in the rat, which can be used effectively for the study of facial paralysis. Specifically, unilateral transection of the VIIth nerve trunk can establish a facial-nerve-palsy model, and subsequent reinnervation with a CFNG can give us information about the process involved that corresponds to the clinical situation. Furthermore, electrophysiologic study of the blink reflex in both the rat and human model yields similar R_1 and R_2 components and, with denervation in both cases, decreased latencies are encountered.

Another recent study in our laboratory has examined the motor end-plate distribution of the normal, denervated and reinnervated orbicularis oculi muscle (OOM) with a CFNG.[33]

Our objective in evaluating the use of a CFNG in treating FP, was to quantitatively ascertain the morphometry of the entire CFNG from its proximal end to its distal coaptation. More specifically, sequential biopsies of the CFNG were examined for the total number of axons, the axon diameter, and the myelin area. Furthermore, this data was correlated with quantitative motor end-plate data, which was the result of our previous report.[32]

MATERIALS AND METHODS

Subjects. Male adult Sprague-Dawley rats (350 to 450 g) were divided into two groups: Group 1—a control group consisting of normal rats that had their left saphenous nerve harvested and analyzed (n = 2); Group 2—an experimental group in which rats received right VIIth nerve transection and then were treated with a CFNG (n = 7). Surgical procedures were performed under general anesthesia (ketamine/xylazine 1 M) and utilizing a Zeiss operating microscope.

Surgical Procedures. *Stage* 1–*Group* A (*control*). The left facial nerve and its branches were explored and dissected via a preauricular incision. Next, the temporal and zygomatic branches to the eye were identified by intraoperative nerve stimulation (Vari-Stim 85-62010). Subsequently, the left saphenous nerve (4.5 to 5.5 cm in length) was harvested for morphometric analysis. Intraoperative photographs and videotapes were taken.

Stage 1–*Group* B (*experimental*). Correspondingly, the left facial nerve trunk and its branches were explored and dissected through a pre-auricular incision. The temporal branch to the eye was identified through intraoperative nerve stimulation. Next, the right facial nerve trunk was explored and transected, producing a complete right facial paralysis. The left saphenous nerve was then harvested as the graft. The proximal end of the saphenous nerve was coapted with the left temporal branch (donor), and tunneled subcutaneously over the dorsum of the cranium to the denervated right side. Coaptations of the saphenous nerve to the temporal branches of the VIIth nerve were performed using an 11-0 suture (Ethilon). Skin closure was accomplished with several 6-0 polypropelene (Prolene) sutures. Again, intraoperative photographs and videotapes were taken.

Stage 2–*Group* B (*experimental*). Three months later, secondary coaptations of the distal end of the saphenous nerve to the right temporal branch were performed.

Stage 3–*Group* B (*experimental*). Three months later, the coaptations of the right-eye temporal branch with the CFNG were explored, and nerve stimulation performed proximal and distal to the coaptation site and in the mid-graft region. The entire graft, along with the proximal and distal coaptations, were then harvested for morphometric analysis.

Histology. Once harvested, specimens were pinned to cork strips with entomology micropins, and fixed in a 4 percent paraformaldehyde, 0.2 percent picric acid solution. Furthermore, each graft was divided into four specimens (PT = proximal temporal branch-donor; PNG = proximal nerve graft; DNG = distal nerve graft; and DT = distal temporal branch-target), rinsed in a 0.1 M cacodylate buffer for 20 min, and post-fixed with 1 percent osmium tetroxide for 2 hr. After dehydration in increasing concentrations of ethanol, the specimens were embedded in resin (Epon 812), cut at 1-micron thickness, mounted on glass slides, and stained with toluidine blue.

MORPHOMETRIC PROCEDURES. Quantitative morphometry was performed using a Zeiss Universal microscope, interfaced with a digitizer tablet (Kurta). Measurements were calculated using the Sigmascan 3.9 software and a 386 computer processor (NEC). All specimens (PT, PNG, DNG, DT, and SN = saphenous nerve biopsy) were compared with respect to the following: a) axon diameter (μm) of each of the nerve fibers; b) myelin sheath area (μm^2) of each of the fibers; c) mean number of axons in each specimen; d) percentage frequency distribution of axonal diameter/specimen type; and e) percentage frequency distribution of myelin area/specimen type.

In studies of developing, mature, and regenerating nerve fibers, both peripheral and central, it has been widely assumed that a single linear relationship exists between myelin-sheath thickness and axon caliber, extending over all fiber-size classes of a given nerve.[34–37] This myelin area-axon caliber relationship was examined in the facial nerve and in the CFNG that was utilized to restore eye-sphincter function in our model. In a recent clinical study, axonal morphometry was studied in FP patients treated with a CFNG and free gracilis muscle transplantation.[38] Our reported results are also discussed in relation to clinical data.

RESULTS

QUANTITATIVE ANALYSIS. *Axon Diameter.* Assessment of the mean axonal diameter of each specimen, using paired t-test comparisons, revealed the following significant differences. Specifically, the proximal temporal (PT) branch-donor showed a significantly greater mean axonal diameter than the proximal nerve graft (PNG; $p < 0.01$), distal nerve graft (DNG; $p < 0.01$), and distal temporal branch-target (DT; $p < 0.05$, Fig. 1). However, the PT branch was not significantly different from the saphenous nerve (SN). In addition, the SN demonstrated significantly greater axon diameter than both PNG and DNG ($p < 0.05$ and $p < 0.02$, respectively).

Myelin Area. Correspondingly, the PT exhibited a significantly greater mean myelin area than the other specimens (Fig. 2). In particular, the PT revealed a greater myelin area than the PNG ($p < 0.01$), DNG ($p < 0.01$), and DT ($p < 0.05$), although no difference was observed between the PT and SN. Analogously, the SN expressed a greater myelin area than both the PNG and DNG ($p < 0.04$ and $p < 0.02$, respectively).

Number of Axons. A comparison of the number of axons between specimens (Fig. 3), using a paired t-test, demonstrated that the DT consisted of a significantly smaller number of axons than the PT ($p < 0.02$). In addition, the DT showed a significantly smaller number of axons than the SN ($p < 0.05$).

Frequency Distribution. The frequency distribution of the myelin area was unimodal for each of the specimens (Fig. 4). Moreover, the distribution demonstrated a shift toward a smaller myelin sheath area (μm^2) per fiber, when comparing the PT with both graft specimens (PNG and DNG) and the DT. Analogously, the axon diameter (μm) frequency distribution for each specimen was illustrated to be unimodal (Fig. 5). Transverse 1-μm thin sections of the entire nerve graft (PT, PNG, DNG, and DT) are illustrated in Figure 6. In addition, a diagrammatic depiction is included indicating the sites of the nerve biopsies (see Fig. 6).

Saphenous Nerve. Comparisons between the saphenous nerve and the proximal temporal branch specimens revealed no statistical difference in the number

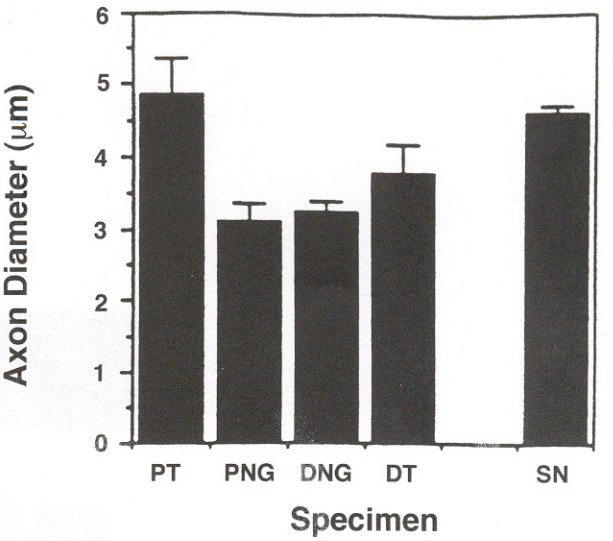

Figure 1. Mean axonal diameter in each specimen. (PT = proximal temporal branch–donor, PNG = proximal nerve graft, DNG = distal nerve graft, DT = distal temporal branch, SN = saphenous nerve.)

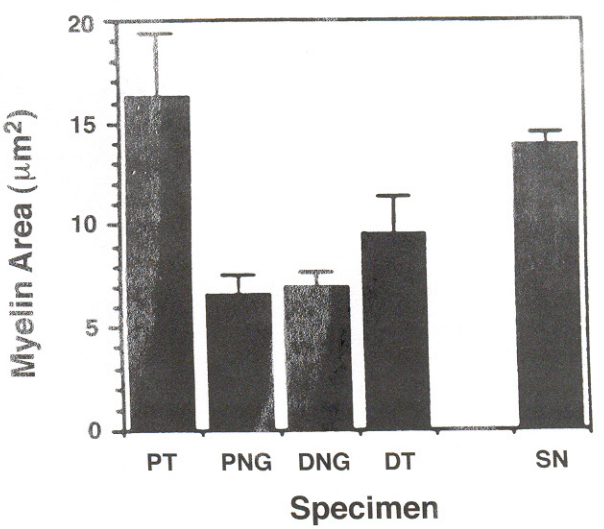

Figure 2. Mean myelin area in each specimen.

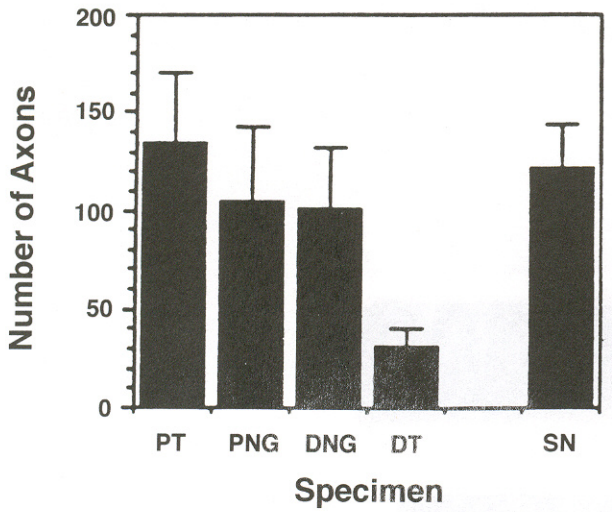

Figure 3. Mean number of axons in each specimen.

of axons, axon diameter, and myelin area (see text above). This histomorphometric compatibility between the donor nerve and the nerve graft strengthens the choice of the saphenous nerve as an optimal nerve graft for this model.

ELECTROPHYSIOLOGIC FINDINGS. Measurements with nerve-conduction velocities of the blink reflex in this model were first reported by Terrell and Terzis,[32] and were correlated with clinical electrophysiologic studies of the blink reflex in man. These data comprised the preoperative quantitative analysis of the blink reflex in the model. The present investigation studied the blink reflex in the reinnervated eye sphincter, and revealed that both the early R_1 and later R_2 responses of the blink reflex were present in 6 of the 7 animals, but that their latencies were slightly decreased for both components.

CORRELATION WITH MOTOR END-PLATE DATA. Motor end-plate analysis of the target muscle (orbicularis oculi) reinnervated by CFNG in this experiment was previously reported.[33] Specifically, the CFNG-treated animals demonstrated a 50 percent increase in the mean number of end plates, compared to the denervated animals. A Spearman correlation test between the number of end plates and the number of axons at the distal temporal level of each animal revealed a positive correlation (0.64).

DISCUSSION

The neuroanatomy of the rat eye sphincter involves two branches of the VIIth nerve (temporal and zygomatic). This enabled our model to "borrow" motor nerve fibers from one of these branches (temporal) on the normal side and subsequently, through the utilization of a CFNG, to reinnervate the contralateral paralyzed eye sphincter.[32]

Quantitative assessment of the entire CFNG procedure revealed a superior axon caliber in the PT segment. Specifically, the PT mean axonal diameter was greater than all of the three more distal nerve segments (PNG, DNG, DT) at 6 months following the initial surgery. These results were in agreement with previous work on CFNGs.[36] However, no difference was observed, when comparing PT with a normal SN specimen. This indicated that, with respect to axon caliber, the saphenous nerve and the temporal branch were compatible, and thus that the SN was a suitable nerve graft. Furthermore, the present results on the temporal branch (PT) of the VIIth nerve correspond very closely with the axon calibers reported by Fraher[37] on the rat oculomotor nerve.

Functionally, the myelin sheath serves as an insulator controlling leakage of current; its area would have to increase accordingly with axon diameter. The present results indicated that myelin area was greatest at the donor site (PT) and significantly smaller at the more distal locations (PNG, DNG, DT). In addition, no statistically significant difference in the myelin area was observed between the PT and the SN, and this provided further support for the suitability of the saphenous nerve as a graft to the facial nerve.

Collectively, the above results demonstrated a positive correlation between myelin area and axon diameter. Although conduction velocity is affected by myelin area, axon diameter and internode length, Brill *et al.*[39] reported that internode length was of secondary importance. More specifically, the effect of internode length was shown to become manifest only for extremely short internodes. In general, axon diameter is thought to be the most important parameter of conduction velocity.[34–36]

Previous research has suggested that regenerated myelinated nerve fibers have thinner sheaths than normal.[35,36,40] Moreover, a comparatively thin sheath is widely accepted as a criterion for identifying a regenerating fiber. In contrast to the PT data, a state of "hypoplasia" of the myelin sheaths in the distal segments (PNG, DNG, DT) of the CFNG may be indicative of insufficient or unsuccessful regeneration. This may have been caused by difficulties associated with carrying out a perfect microsurgical coaptation, due to the tiny size of the VIIth nerve branches in this model (<0.01 mm for the temporal branch, despite the use of high magnification). Furthermore, an impaired regenerative capacity of the Schwann cell for myelin formation, or an ineffective communication between the axon and the Schwann cell, may be involved.[41]

When examining the mean number of axons present throughout the CFNG, a significant decrease was noted in the DT specimens. This may suggest that there was a direct relationship between axon diameter, myelin area, and the number of fibers in the distal end of the CFNG. This was in contrast to the findings

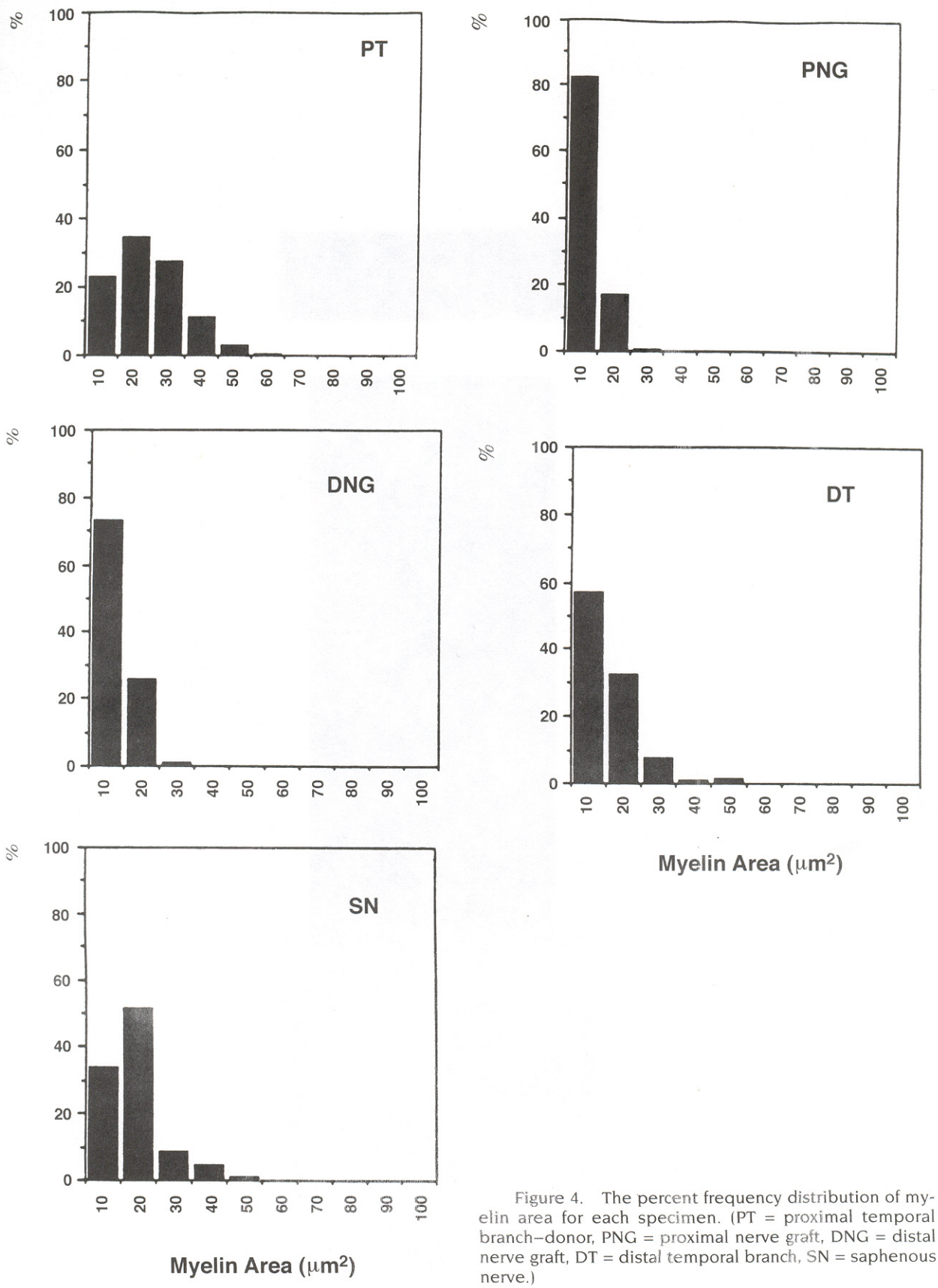

Figure 4. The percent frequency distribution of myelin area for each specimen. (PT = proximal temporal branch–donor, PNG = proximal nerve graft, DNG = distal nerve graft, DT = distal temporal branch, SN = saphenous nerve.)

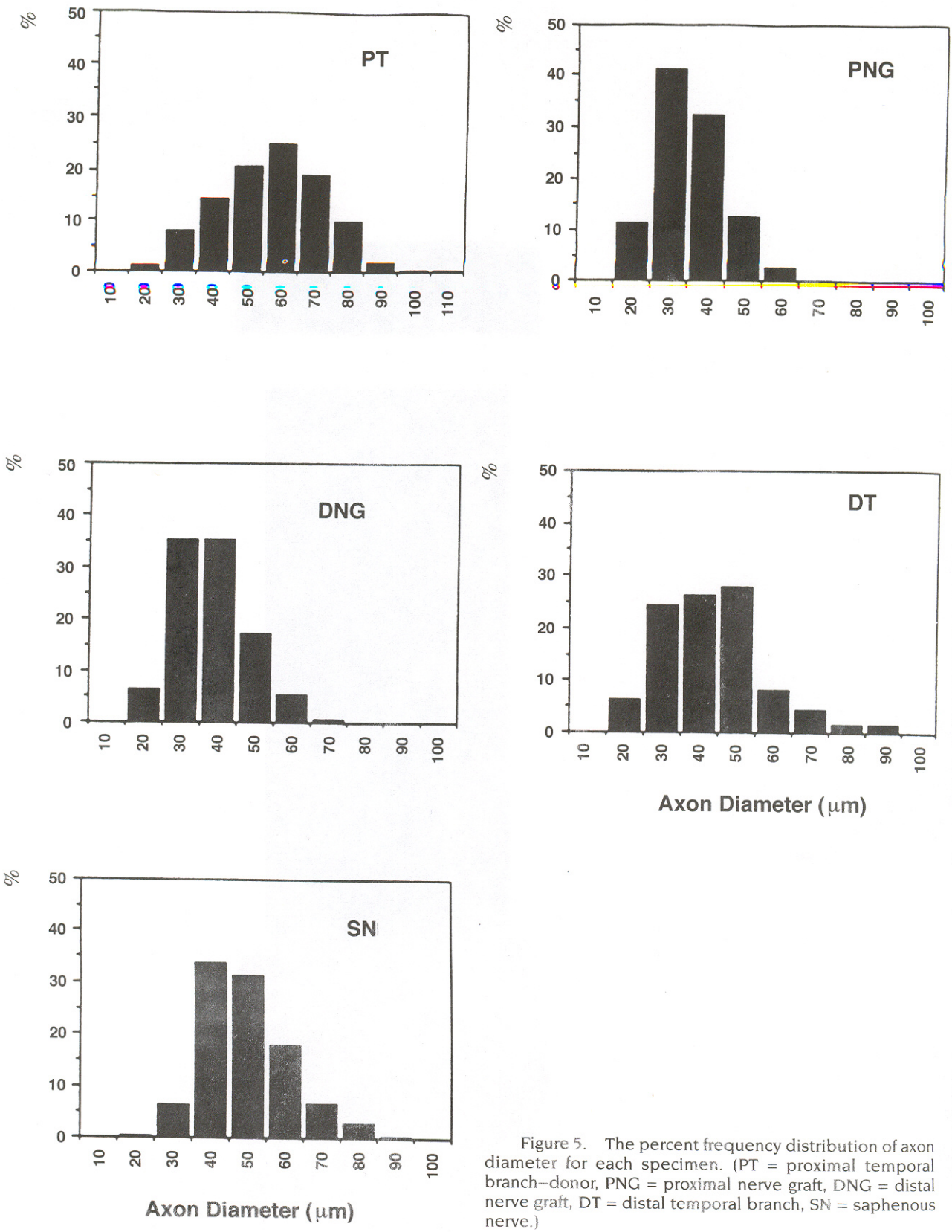

Figure 5. The percent frequency distribution of axon diameter for each specimen. (PT = proximal temporal branch–donor, PNG = proximal nerve graft, DNG = distal nerve graft, DT = distal temporal branch, SN = saphenous nerve.)

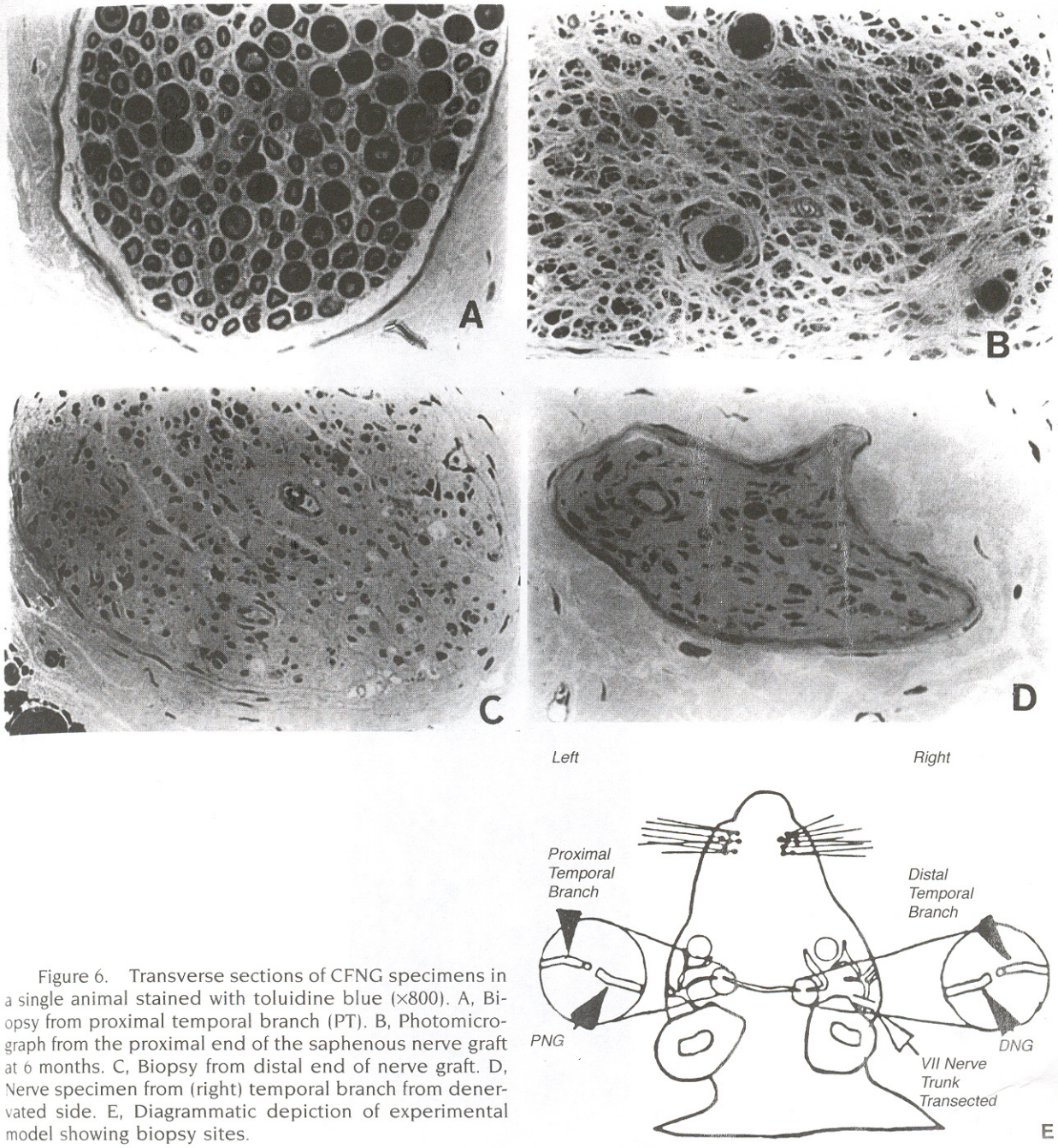

Figure 6. Transverse sections of CFNG specimens in a single animal stained with toluidine blue (×800). A, Biopsy from proximal temporal branch (PT). B, Photomicrograph from the proximal end of the saphenous nerve graft at 6 months. C, Biopsy from distal end of nerve graft. D, Nerve specimen from (right) temporal branch from denervated side. E, Diagrammatic depiction of experimental model showing biopsy sites.

of Frey *et al.*,[38] who reported that, regardless of function, the same number of regenerated, thin, nerve fibers were found in the distal segments of a CFNG at the time of clinical muscle transplantation.

A critical element in conducting morphometric analyses of nerves is to examine the frequency distribution of both axon diameter and myelin area. Results illustrated a shift toward thinner myelin areas, and this pattern was in agreement with previous clinical and experimental CFNG studies.[36,38] The distal graft (DT) showed a concentration of only a few classes of myelin area, with a strong shift to the left toward thinner myelinated fibers. The exact correlation of these fibers with functional recovery has not been determined. In addition, the unimodal frequency distribution of axon diameter was consistent with a previous study using the sural nerve as a CFNG to treat FP.[38] Despite a small shift toward smaller-diameter axons in the DT specimens, the shape of the distribution between PT and DT was very similar.

Finally, the present results were compared to the OOM motor end-plate data in the same animals. In particular, the relationship was studied between the number of motor end plates in the target (OOM) and the number of axons found in the distal temporal branch of the CFNG. The comparison indicated an

almost 1:1 ratio. This may suggest that recovery of eye-sphincter function bears a linear relationship to the number and condition of axons that have successfully reached the distal end of the CFNG. Future attempts at reanimating the eye sphincter should emphasize CFNG continuity, with the provision of a well-vascularized and trophic environment.

Currently the CFNG procedure is extensively utilized in humans with FP. As a matter of fact, it is the only procedure available that provides the anatomic and physiologic substrates for emotional facial expression and voluntary animation. Estimates indicate that thousands of cases per year of facial paralysis in the U.S. alone could benefit from the use of CFNG techniques.[42] In conclusion, the present histomorphometric analysis of the CFNG in the rat model provides further insight into the neural dynamics for guiding microsurgical restoration of the blink reflex. More important, these data can be correlated with clinical results and can facilitate the development of methods better capable of restoring eye-sphincter function. Of course, a problem in reestablishing facial symmetry with CFNG techniques is the possibility of an atrophic OOM. Current research in our laboratory is addressing this problem, by combining a CFNG in our model with provision of a new homologous muscle target.

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